

computer readable form, and a paper copy of the sequence information that has been printed from the floppy disk.

The information contained in the computer readable form (floppy disk) was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy.

This amendment contains no new matter. The amendments to the specification and/or claims are to provide a formal sequence listing and/or to provide appropriate cross-references to SEQ ID Numbers in accordance with 37 C.F.R. §§1.821 to 1.825. The sequence information provided herein finds support in the specification as filed.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

LAW OFFICES OF JONATHAN ALAN QUINE  
P.O. BOX 458  
Alameda, CA 94501  
Tel: 510 337-7871  
Fax: 510 337-7877

Respectfully submitted,

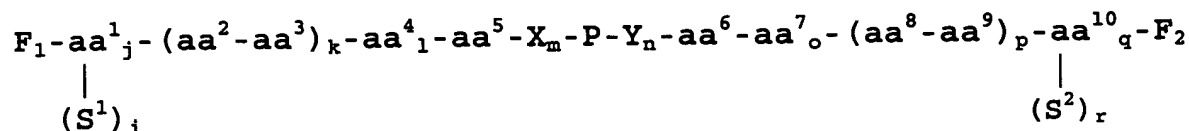


Tom Hunter  
Attorney for Applicant(s)  
Reg. No: 38,498

## APPENDIX A

## MARKED UP VERSION OF ALTERED CLAIMS SHOWING AMENDMENTS.

1. A fluorogenic composition for the detection of the activity of a protease, said composition having the formula:



wherein, P is a peptide selected from the group consisting of DEVDGIN (SEQ ID NO:193), (d-O)DEVDGIN (SEQ ID NO:194), DEVDGID (SEQ ID NO:195), LVEIDNG (SEQ ID NO:196), GIETESGV (SEQ ID NO:197), TGRT (SEQ ID NO:198), VMTGRT (SEQ ID NO:199), SEVKLDAEF (SEQ ID NO:200), S(d-E)VK(d-L)DAE(d-F) (SEQ ID NO:201), EDVVCCS (SEQ ID NO:202), EEVEGIN (SEQ ID NO:203), D(d-F)VDGIN (SEQ ID NO:204), (d-D)EV(d-D)GIN (SEQ ID NO:205), LVEIENG (SEQ ID NO:206), GIETDSG (SEQ ID NO:207), GIETESG (SEQ ID NO:208), LEHDGIN (SEQ ID NO:209), LETDGIN (SEQ ID NO:210), WEHDGIN (SEQ ID NO:211), YVHDG (SEQ ID NO:212), YVHDGIN (SEQ ID NO:213), YVHDA (SEQ ID NO:214), TGRTG (SEQ ID NO:215), S(d-E)VK(d-L)DAE(d-F) (SEQ ID NO:216), IEPDS (SEQ ID NO:217), PLGIAGI (SEQ ID NO:218), SQNYPIVQ (SEQ ID NO:219);

F<sup>1</sup> and F<sup>2</sup> are fluorophores and F<sup>1</sup> is attached to the amino terminal amino acid and F<sup>2</sup> is attached to the carboxyl terminal amino acid;

S<sup>1</sup> and S<sup>2</sup>, when present, are peptide spacers ranging in length from 1 to about 50 amino acids and S<sup>1</sup>, when present, is attached to the amino terminal amino acid and S<sup>2</sup>, when present, is attached to the carboxyl terminal amino acid;

i, j, k, l, m, n, o, p, q, and r are independently 0 or 1;

aa<sup>1</sup> and aa<sup>10</sup> are independently selected from the group consisting of lysine, ornithine and cysteine;

aa<sup>2</sup>, aa<sup>3</sup>, aa<sup>8</sup>, and aa<sup>9</sup> are independently selected from the group consisting of an amino acid or a dipeptide consisting of Asp, Glu, Lys, Ornithine, Arg, Citulline, homocitrulline, Ser, homoserine, Thr, and Tyr;

$aa^5$ ,  $aa^4$ ,  $aa^6$ , and  $aa^7$  are independently selected from the group consisting of proline, 3,4-dehydropyrrolidine, hydroxyproline, alpha aminoisobutyric acid and N-methyl alanine;

X is selected from the group consisting of Gly,  $\beta$ Ala,  $\gamma$ Abu, Gly-Gly, Ahx, C7,  $\beta$ Ala-Gly,  $\beta$ Ala- $\beta$ Ala,  $\gamma$ Abu-Gly,  $\beta$ Ala- $\gamma$ Abu, Gly-Gly-Gly,  $\gamma$ Abu- $\gamma$ Abu, Ahx-Gly,  $\beta$ Ala-Gly-Gly, Ahx- $\beta$ Ala,  $\beta$ Ala- $\beta$ Ala-Gly, Gly-Gly-Gly-Gly (SEQ ID NO:220), Ahx- $\gamma$ Abu,  $\beta$ Ala- $\beta$ Ala- $\beta$ Ala,  $\gamma$ Abu- $\beta$ Ala-Gly,  $\gamma$ Abu- $\gamma$ Abu-Gly, Ahx-Ahx,  $\gamma$ Abu- $\gamma$ Abu- $\beta$ Ala, and Ahx-Ahx-Gly;

Y is selected from the group consisting of Gly,  $\beta$ Ala,  $\gamma$ Abu, Gly-Gly, Ahx, C7, Gly- $\beta$ Ala,  $\beta$ Ala- $\beta$ Ala, Gly- $\gamma$ Abu,  $\gamma$ Abu- $\beta$ Ala, Gly-Gly-Gly,  $\gamma$ Abu- $\gamma$ Abu, Gly-Ahx, Gly-Gly- $\beta$ Ala,  $\beta$ Ala-Ahx, Gly- $\beta$ Ala- $\beta$ Ala, Gly-Gly-Gly-Gly,  $\gamma$ Abu-Ahx,  $\beta$ Ala- $\beta$ Ala- $\beta$ Ala, Gly- $\beta$ Ala- $\gamma$ Abu, Gly- $\gamma$ Abu- $\gamma$ Abu, Ahx-Ahx,  $\beta$ Ala- $\gamma$ Abu- $\gamma$ Abu, and Gly-Ahx-Ahx;

when i is 1,  $S^1$  is joined to  $aa^1$  by a peptide bond through a terminal alpha amino group of  $aa^1$ ; and when r is 1,  $S^2$  is joined to  $aa^{10}$  by a peptide bond through a terminal alpha carboxyl group of  $aa^{10}$ .

4. The composition of claim 1, having an amino acid sequence selected from the group consisting of Fa-KDPJGDEVVDGNGJPKGY (SEQ ID NO:221), Fm-KDPJGDEVVDGNGJPKamide (SEQ ID NO:222), Fm-KDPJG (d-O)DEVVDGNGJPKGY (SEQ ID NO:223), Fm-KDPJGDEVVDGNGJPKGY (SEQ ID NO:224), Fm-KDPGDEVVDGNGJPKGY (SEQ ID NO:225), Fm-KDPJGDEVVDGIDGJPKamide (SEQ ID NO:226), Fm-KDPJGLVEIDNGJPKGY (SEQ ID NO:227), Fm-KDPJGIETESGVGJPKGY (SEQ ID NO:228), Fm-KDPJTGRTGPKGY (SEQ ID NO:229), Fm-DPTGRTGPKGY (SEQ ID NO:230), Fm-KDPVMTGRTGJPKGY (SEQ ID NO:231), Fm-KDPTGRTGJPKGY (SEQ ID NO:232), Fm-KDPJGTGRTGJPKGY (SEQ ID NO:233), Fm-KDPJGTGRTGPKGY (SEQ ID NO:234), Fm-KDPGTGRTGPKGY (SEQ ID NO:235), Fm-KDPJGSEVKLDAEFGJPKGY (SEQ ID NO:236), Fm-KDPJGS (d-E)VK (d-L)DAE (d-F)GC5PKDDY (SEQ ID NO:237), Fa-KDPJGEDVVCCSGJPKGY (SEQ ID NO:238), KDPJGEEVEGINGJPKGY (SEQ ID NO:239), KDPJGD (d-F)VDGNGJPKGY (SEQ ID NO:240), KDPJG (d-D)EV (d-D)GINGJPKGY (SEQ ID NO:241), KDPJGLVEIENGJPKGY (SEQ ID NO:242), KDPJGIETDSGJPKGY (SEQ ID NO:243), KDPJGIETESGJPKGY (SEQ ID NO:244), KDPJGLEHDGNGJPKGY (SEQ ID NO:245), KDPJGLETGNGJPKGY (SEQ ID NO:246), KDPJGWEHDGNGJPKGY (SEQ ID NO:247), KDPJGYVHDGJPKGY (SEQ ID NO:248), KDPJGYVHDGNGJPKGY (SEQ ID NO:249), KDPJGYVHDAPKGY (SEQ ID NO:250),

KDPJTGRTGJPKG Y (SEQ ID NO:251), KDPC3TGRTGPKG Y (SEQ ID NO:252),  
KDPC7TGRTGPKG Y (SEQ ID NO:253), KDPC5GS(d-E)VK(d-L)DAE(d-F)GJPKG Y (SEQ ID  
NO:254), KDPJGIEPDSGJPKG Y (SEQ ID NO:255), KDPJGPLGIAGIGJPKG Y (SEQ ID NO:256),  
and KDPJGSQNYPIVQGJPKG Y (SEQ ID NO:257).

**APPENDIX B****MARKED UP VERSION OF ALTERED PARAGRAPHS SHOWING AMENDMENTS TO SPECIFICATION.**

New Tables 3, 4, and 12 are amended to provide cross-referencing for SEQ ID Numbers.

Page 37, lines 15-22:

--When it is desired to link the indicator to a solid support through the peptide backbone, the peptide backbone may comprise an additional peptide spacer (designated S<sup>1</sup> or S<sup>2</sup> in Formula I). The spacer may be present at either the amino or carboxyl terminus of the peptide backbone and may vary from about 1 to about 50 amino acids, more preferably from 1 to about 20 and most preferably from 1 to about 10 amino acids in length. Particularly preferred spacers include Asp-Gly-Ser-Gly-Gly-Gly-Glu-Asp-Glu-Lys (SEQ ID NO:161), Lys-Glu-Asp-Gly-Gly-Asp-Lys (SEQ ID NO:162), Asp-Gly-Ser-Gly-Glu-Asp-Glu-Lys (SEQ ID NO:163), and Lys-Glu-Asp-Glu-Gly-Ser-Gly-Asp-Lys (SEQ ID NO:164).--

Page 52, lines 13-21:

--Fluorophores were linked to the amino terminus via the  $\alpha$ -amino group of Aspartic acid residue (D) and to the  $\epsilon$ -amino group of lysine (K). Labeling was accomplished by the displacement of a succinimidyl group linked to 6-TMR or DER. The structure of the peptide, called NorFES-KGY is:

**Fluorophore1-DAIPNleSIPKGY**

|

**Fluorophore2**

(SEQ ID NO:165)--

Page 55, lines 7-15:

--In addition, we have synthesized and derivatized (homodoubly-labeled) PAI-2, CS-1 (a 31 residue long peptide) and two DEVD-like peptides that did not allow the dye-dye dimer formation. The CS-1 peptide shows that in a significantly longer peptide the dye-dye dimer structure can be formed. Note this peptide contains four proline residues in the amino terminal side of the putative cleavage site Ile-Leu bond. There is one proline in the carboxyl domain also. The results from the CS-1 peptide support a potentially larger sequence between the two dyes (fluorophores). Two DEVD-like

peptide's amino acid sequences that did not allow the formation of productive H-type dimers are F<sub>1</sub>-DEVGDIDPK[F<sub>1</sub>]GY (SEQ ID NO:166) and F<sub>1</sub>-PDEVGDIDPK[F<sub>1</sub>]GY (SEQ ID NO:167).--

Page 57, lines 10-21:

-- The elastase substrate, Fm-K[F<sub>1</sub>]DAIPNluSIPK[F<sub>1</sub>]GY, (SEQ ID NO:182), where F<sub>1</sub> was carboxytetramethylrhodamine, Fm was Fmoc, K[F<sub>1</sub>] was F<sub>1</sub> covalently attached through the epsilon amino group of lysine (K), and Fm-K is the Fmoc group covalently attached at the alpha amino group of the amino terminal lysine residue) was used with HL-60 cells. Cells were incubated with various concentrations of elastase substrate ranging from 10 nM to 10 µM for 5 minutes to 60 minutes. Then the cells were diluted 5-fold with RPMI 1640 medium containing 5% serum or with phosphate buffered saline. The samples were centrifuged and washed once more with 1 ml of washing solution. After centrifugation and removal of the washing solution, cell pellets were loosened with about 25 µl of medium and these cells were transferred to a glass capillary. Capillary tubes were then placed on a glass microscope slide and examined under a fluorescence microscope using standard rhodamine filters.--

Page 58, lines 6-23:

--Control cells without substrate incubation and the sample with the greatest expected fluorescence signals were used to set the instrument detector parameters. For example after 15 minutes incubation of Jurkat cells with substrate compound #11 Fm-CGD2D: Fm-K[F<sub>1</sub>]DBJGDEVGDIDGJPK[F<sub>1</sub>]GY (SEQ ID NO:183, where F<sub>1</sub> was carboxytetramethylrhodamine; Fm was Fmoc, K[F<sub>1</sub>] was F<sub>1</sub> covalently attached through the epsilon amino group of lysine (K), Nlu was norleucine, B was aminoisobutyric acid, and J was epsilon-aminocaproic acid) an increase of about 10 channels indicating cellular uptake of the substrates was measured. Note substrate #11 was not completely quenched. Hence, a small amount of background fluorescence would be expected from the intact substrate. Signals from the cells that had been activated with 1 µg/ml of anti-Fas antibody, CH11 clone for 1 to 6 hours indicated an increase in peak channel number. As much as a ten-fold increase in fluorescence intensity was observed. When the cells were co-incubated with the CPP32 protease inhibitor ZVAD-fluoromethylketone at 50 µM along with an apoptosis inducing agent, *e.g.*, anti-Fas antibody, this observed increase in fluorescence intensity was eliminated. This indicated that the signal from compound 11 was due to the CPP32 protease activity which was inhibitable by ZVAD-FMK.

Hence, the observed fluorescence intensity in each cell as determined by flow cytometric analysis served as a direct measure of the intracellular CPP32 protease activity.--

Page 59, lines 18-27:

--Jurkat cells are normally grown in 10% fetal calf serum containing RPMI 1640, at 37° C in a 5% CO<sub>2</sub> atmosphere. When the serum content was dropped to 4%, the Jurkat cell growth rate not only slowed down but also a significant number of cells died within 36 hours. The cell density used was about 400,000 cell per ml. After 36 hours, control wells contained about 50% dead cells (trypan blue-positive cells), whereas the wells containing 0.1 or 1.0 µM concentration of compound #11 (Table 12) "Fm-CGD2D" or Fm-K[F1]DBJGDEVGDGIDGJPK[F1]GY (SEQ ID NO:184) showed only 10% or 8% nonviable cells. Hence, compound #11 which exhibits efficient cellular uptake slowed down apoptosis in these Jurkat cells where it acted as a CPP32 protease inhibitor or a CPP32 activating protease inhibitor. --

Page 61, line 26 - page 63, line 15:

-- The parent compound Fm-DEVD has the following composition: Fmoc-K[F1]DB**DEVD**GIDPK[F1]GY (SEQ ID NO:185). The bold face underlined letters are the protease recognition sequence consisting of 7 amino acid residues. Compound #10 contains two glycine extensions at both ends of this protease recognition sequence. The central protease recognition domain now is 8 residues long GDEV**DGID** (SEQ ID NO:186), since the glycine residue at the amino terminus is a part of native sequence. The two glycine residues which are inherently more flexible than other amino acids, *e.g.*, alanine, provide less conformational constraint or, conversely, more flexibility than compound 4 (Table 12) and thereby permit greater flexion when combined with Aib or Pro residues. Additional insertion of amino caproic acid at both termini with five methylene groups in addition to the one present in glycine provides further relaxation of the constrained conformation and, thus, greater flexibility for the protease recognition domain, GDEV**DGID** (SEQ ID NO:186). This progression of flexibility resulted in an increased hydrolysis rate with the CPP32 protease since CPP32 recognizes a more flexible protease recognition domain than does elastase. Support for this statement is that the CPP32 protease cleavage site in the proform of its physiological substrate, poly(ADP-ribose) polymerase, PARP, is located between two well-folded domains. Hence, it is expected that such a protease cleavage site would not be rigidly held or its conformation would be expected to be less defined

than the remaining molecule. Hence, in order to provide these structural features to the substrate, introduction of flexible residues such as glycine, epsilon amino caproic acid, beta alanine, and amino butyric acid would be expected to play important roles in regulating the backbone flexibility of the substrate's central protease recognition domain. These additional preferred residues for the conformation determining domain are also expected to provide the needed bend-inducing influence.--

Page 62, lines 23-29:

--These examples provide a tetrapeptide and a pentapeptide comprising Lys-Asp-Aib-Gly (SEQ ID NO:187) or Lys-Asp-Aib-Ahx-Gly (SEQ ID NO:188) where Ahx is epsilon amino caproic acid (*i.e.*  $\text{NH}_2-(\text{CH}_2)_5-\text{COOH}$ ). The fluorophore is attached to epsilon amino group of the lysine residue. The carboxyl terminal CDR domain is defined as a tripeptide Gly-Pro-Lys and a tetrapeptide Gly-Ahx-Pro-Lys (SEQ ID NO:189). The hydrolysis rate was increased by 3-fold between compounds 4 (Fm-DEVD: Fm-K[F1]DBDEVGDGIDPK[F1]GY, SEQ ID NO:190) and 10 (Fm-G2D2D: Fm-K[F1]DBGDEVGDGIDGPK[F1]GY, SEQ ID NO:191).--

Page 62, lines 30-34:

--As illustrated in Figure 5, the hydrolysis rate was further increased by *ca.* 3-fold over the above glycine residue insertion with the amino caproic amino acid (Ahx) addition, compound 11 (Fm-CGD2D: Fm-K[F1]DB Ahx GDEVGDGIDG Ahx PK[F1]GY, SEQ ID NO:192). Hence, overall at least a 9-fold increase in substrate hydrolysis rate was accomplished (compounds 4 and 11, Table 12).--